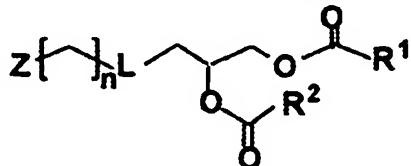


Amendments to the Specification

On page 4, please replace the paragraph starting on line 13 with the following:

Accordingly, in one aspect, the invention includes a composition for administration of a nucleic acid, comprising

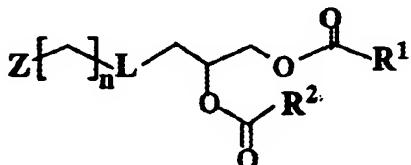
(a) liposomes comprised of (i) a lipid having the formula:



A1
where each of R¹ and R² is an alkyl or alkenyl chain having between 8-24 carbon atoms, and each of R¹ and R² are independently selected; n = [1]0-20; L is selected from the group consisting of (1) -X-(C=O)-Y-[[CH₂-]], (2) -X-(C=O)-, and (3) -X-[[CH₂-]], where X and Y are independently selected from oxygen, NH and a direct bond; Z is a weakly basic moiety that has a pK of less than 7.4 and greater than about 4.0.

On page 10, please replace the paragraph starting on line 19 with the following:

The cationic-neutral lipid included in the liposomes of the present invention is generally a lipid represented by the following structure:



A2
where each of R¹ and R² is an alkyl or alkenyl chain having between 8-24 carbon atoms; n = [1]0-20 and in a preferred embodiment is between 1-10; L is selected from the group consisting of (i) -X-(C=O)-Y-[[CH₂-]], (ii) -X-(C=O)-, and (iii) -X-[[CH₂-]], where X and Y are independently selected from oxygen, NH and a direct bond; and Z is a weakly basic moiety that has a pK of less than 7.4 and greater than about 4.0.

On page 13, please replace the paragraph starting on line 4 with the following:

AB
Also included as embodiments of Z are certain acyclic amine compounds, such as N,N'-dimethylguanidin and various substituted hydrazines, such as trimethylhydrazine, tetramethylhydrazine, 1-methyl-1-phenylhydrazine, 1-naphthalenylhydrazine, and 2-, 3-, and 4-methylphenyl hydrazine, all of which are reported to have pKa' between 4.5 and 7.0. Alicyclic compounds having pKa's in this range include 1-pyrrolidineethanamine, 1-piperidineethanamine, hexamethylenetetramine, and 1,5-diazabicyclo[3.3.3]undecane.

On page 13, please replace the paragraph starting on line 24 with the following:

AH
The lipids of the invention include a neutral linkage L joining the Z moiety and the tail portion of the lipid. Linkage L is variable, and in preferred embodiments is selected from a carbamate, an ester, an urea amide, a carbonate, a urea, an amine, and an ether. In a preferred lipid prepared in support of the invention, a carbamate linkage, where L is -X-(C=O)-Y-[CH₂-], X being NH and Y being oxygen, was prepared.

On page 14, please replace the paragraph starting on line 4 with the following:

AS
The lipid of the invention can be prepared using standard synthetic methods. As mentioned above, in studies performed in support of the invention, a lipid having the structure shown above, where Z is an imidazole, $[\text{N}]_n = 2$, L is a carbamate and $R^1 = R^2 = C_{17}H_{35}$, was prepared. A reaction scheme for preparation of the exemplary lipid is illustrated in Fig. 1 and details of the synthesis are provided in Example 1. Briefly, the *para*-nitrophenyl carbonate of 1,2-distearoyl glycerol (Compound III) was prepared from 1,2-distearoyl-sn-glycerol (Compound I) and *para*-nitrophenyl chloroformate (Compound II) and reacted with histamine (Compound IV) to yield a lipid (Compound V) having a imidazole moiety linked to a distearoyl tail via a carbamate linkage. A similar route, using glycerol in place of 1-amino-2,3-propanediol, can be used to produce a carbonate-linked product (L = -O-(C=O)-O-CH₂-).

On page 14, please replace the paragraph starting on line 19 with the following:

AB Preparation of the lipid having other linkages is readily done by those of skill in the art using conventional methods. Other linkages include ether ($L = -O-CH_2-$) and ester linkages ($L = -O-(C=O)-$), as well as urea amide, urea and amine linkages (i.e., where $L = -NH-(C=O)-NH-$, $-NH-(C=O)-[CH_2-]$, $[-NH-(C=O)-NH-CH_2-]$, or $-NH-[CH_2-]$). A keto linkage, where $[(L)]X$ is a direct bond, is also possible. Figs. 2A-2B illustrate preparation of an etheramine-linked lipid (Fig. 2A) and an ester-linked lipid having an NH-containing linkage (Fig. 2B). In Fig. 2A, the terminal amine of histamine is reacted with ~~with~~ glycidyl chloride, the resulting epoxide is hydrolyzed, and the resulting diol is acylated.

On page 14, please replace the paragraph starting on line 30 with the following:

A7 In Fig. 2B, an ester-linked lipid having an NH-containing linkage ($L = -O-(C=O)-CH_2-$) is prepared, for example, by reacting histamine with an activated derivative of glyceric acid acetonide (2,2-dimethyl-1,3-dioxolane-4-carboxylic acid) or the four-carbon homolog, 2,2-dimethyl-1,3-dioxolane-4-acetic acid, as shown. The diol is then deprotected and acylated.

On page 15, please replace the paragraph starting on line 3 with the following:

AB Figs. 2C and 2D show other reaction schemes for preparation of pH-responsive lipids in accord with the invention. In Fig. 2C, 4-nitrobenzoic acid is condensed with 1-amino-2,3-propanediol, giving an amide linkage; the diol is acylated and the nitro group reduced to an amine to give the product, a lipid-aniline conjugate. In Fig. 2D, the initial condensation reaction is between an alcohol (diacylglycerol) and an isocyanate, giving a carbamate linkage in the product.

On page 15, please replace the paragraph starting in line 3 with the following:

AG Figs. 3A-3D show various structures of pH-responsive lipids in accord with the invention, where Figs. 3A-3B show further lipids having an aromatic amine as the "Z" moiety, and Figs. 3C-3D show lipids having an aminosugar attached to a lipid.

AG

Synthesis of these lipids can be readily performed by those of skill in the art using commercially available starting materials. For example, the product of Fig. 3A may be prepared by reaction of m-nitrobenzyl bromide and a diacylglycerol, giving the ether linkage, followed by reduction of the nitro group. The product of Fig. 3B is prepared from commercially available (2-nitrophenyl)-1,2-ethanediol by acylation of the diol and reduction of the nitro group. To prepare the aminosugar-lipid conjugate shown in Fig. 3C, D-glucose (furanose form) is protected by reaction with two molecules of acetone, and the free hydroxyl group is sequentially reacted with TsCl, sodium azide, and iodine to give an intermediate nitro compound. The exocyclic diol is deprotected and acylated, and the nitro group reduced to the amine. The compound of Fig. 3D can be prepared in a similar manner from D-galactose.

AJD

On page 18, please replace the paragraph starting on line 21 with the following:

In this reaction scheme, mPEG-methyl-dithiobenzyl- nitrophenyl chloroformate was reacted with DSPE to form the desired compound. The nitrophenyl chloroformate moiety in the mPEG-methyl-dithiobenzyl-nitrophenyl chloroformate compound acts as a leaving group to yield the desired product upon reaction with a selected lipid. The compound can also be produced by reaction with a compound such as mPEG-methyl-dithiobenzyl-R³, where R³ represents a leaving group joined through a linking moiety to the benzene ring. The leaving group is displaced upon reaction with an amine-containing ligand, such as DSPE, a polypeptide or an amine-containing drug. The leaving group is selected according to the reactivity of the amine in the ligand, and is preferably derived from various acidic alcohols that have a hydroxy- or oxy-containing leaving group. These include chloride, p-nitrophenol, o-nitrophenol, N-hydroxy-tetrahydrophthalimide, N-hydroxysuccinimide, N-hydroxy-glutarimide, N-hydroxynorbornene-2,3-dicarboxyimide, 1-hydroxybenzotriazole, 3-hydroxypyridine, 4-hydroxypyridine, 2-hydroxypyridine, 1-hydroxy-6-trifluoromethylbenzotriazole, imidazole, triazole, N-methyl-imidazole, pentafluorophenol, trifluorophenol and trichlorophenol.

On page 27, please replace the paragraph starting on line 21 with the following:

A/1

Examples 8-12 describe other studies performed in support of the invention, where FGF-targeted liposome/DNA complexes were administered to mice inoculated with Lewis lung tumors or to mice injected with Matrigel, an FGF-angiogenic endothelial cell model for tumor vasculature targeting. In these studies, liposomes in accord with the invention were composed of the neutral-cationic lipid HDSG (Compound V of Fig. 1) and either cholesterol or PHSPC. PEG-DTB-lipid was also included in the formulations in accord with the invention. A cationic lipid was also included in the complexes, to determine the effect of the cationic lipid on complex stability and transfection efficiency. Two cationic lipids were utilized, DOTAP and N²-[N², N⁵-bis(3-aminopropyl)-L-ornithyl]-N,N-di octadecyl-L-glutamine tetrahydrotrifluoroacetate, referred to herein as "GC33".

A/2

On page 30, please replace Table 5 with the following:

Formulation No. (See Example 11 for details)	Targeting Ligand	Luciferase Expression (pg luciferase/mg protein)		
		Matrigel	Lung	Liver
Formulation No. 11-1 (DOTAP/Chol)	none	35.0	102929.3	116.9
Formulation No. 11-2 (HDSG/PHSPC)	FGF	34.3	1.6	2.1
Formulation No. 11-3 (HDSG/DOTAP/PHSPC)	FGF	45.5	1.7	2.3
Formulation No. 11-4 (HDSG/DOTAP/CHOL)	none	40.9	62.0	3.0
Formulation No. 11-5 (HDSG/DOTAP/CHOL)	FGF	21.4	61.2	4.0
Formulation No. 11-6 (HDSG/DOTAP/CHOL/FC- PEG)	FGF	64.5	4.0	1.6
Formulation No. 11-7 (HDSG/DOTAP/CHOL)	Sialyl Lewis ^x	42.8	34.1	2.2
Formulation No. 11-8 (HDSG/DOTAP/CHOL)	FGF	38.1	12.4	2.6

On pag 30, please r place Tabl 6 with the following:

AB

Formulation No. (See Example 12 for details)	Targeting Ligand	Luciferase Expression (pg luciferase/mg protein) ¹		
		Tumor	Lung	Liver
Formulation No. 12-1 (DOTAP/Chol)	none	72.1	273214.0	174.4
Formulation No. 12-2 (HDSG/PHSPC) (>5 days)	FGF	17.5	9.2	3.5
Formulation No. 12-3 (HDSG/PHSPC) (1 day)	FGF	32.8	8.4	3.9
Formulation No. 12-4 (HDSG/DOTAP/Chol/FC-PEG) (>5 days)	none	22.3	12.2	6.2
Formulation No. 12-5 (HDSG/DOTAP/Chol/FC-PEG) (1 day)	FGF	33.4	23.2	2.5

¹expression was measured 24 hours after liposome administration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4.

On page 30, please replace the paragraph starting on line 11 with the following:

AIY

The data in Tables 2-6 show that using the HDSG neutral-cationic lipid rather than a conventional cationic lipid achieved an extended biodistribution of the DNA-liposome complexes. This feature can be seen by comparing, for example, the luciferase expression in the lung and tumor for [if] Formulation [in] Nos. 9-1 and 9-2. This enhanced biodistribution achieved when the neutral cationic lipid was included in the liposomes even when a cationic lipid was present in addition to the neutral-cationic lipid, as seen when the results for Formulation Nos. 10-1 and 10-2 are compared.

On page 31, please replace the paragraph starting on line 8 with the following:

AIS

The effect of the FGF targeting ligand on *in vivo* gene expression can be seen by comparing Formulation Nos. 9-2 with 9-3 and Formulation Nos. 9-5 with 9-6 and Formulation Nos. 10-3 with 10-4. In these formulations, luciferase expression was higher, and sometimes significantly higher, when the complexes included the FGF targeting ligand.

On page 31, please replace the paragraph starting on line 14 with the following:

AK

A comparison of Formulation Nos. 9-7, 9-8, and 9-9 with Formulation Nos. 10-5, 10-6, and 10-7 show that reducing the amount of GC33 from 22.5 mole percent to

A16
11.25 mole percent shifts the luciferase expression from the lung to the tumor or Matrigel. This shift of expression level 1 is due to a reduction in surface charge of the liposome-DNA complexes, indicating that the absence of surface charge at physiological pH increases blood retention time and changes the tissue distribution of the complexes after systemic administration.

A17
On page 31, please replace the paragraph starting on line 23 with the following:

Examples 13-15 describe *in vivo* administration of various liposome formulations to mice bearing a KB tumor. In these studies, the complexes included a folate ligand for targeting to the folate-expressing tumor cells. The formulations are described in Example 13-15 and the luciferase/luciferase expression in the tumor lung and liver following intravenous administration are shown in Table 7-9.

A18
On page 44, please replace the paragraph starting on line 10 with the following:

3. 1-(mercaptomethyl)ethylammonium chloride. 5-Methylthiazolidine-2-thione (6.5 g, 49 mmol) was placed in a 250 ml round-bottom-flask. A solution of aqueous hydrochloric acid (40 ml, 18% in H₂O) was added and the flask was heated in an oil bath. The reaction refluxed (120°C) for one week. Three times throughout the week 1 ml of concentrated hydrochloric acid was added. The reaction was monitored using TLC with ethyl acetate as eluent. They were visualized using UV, ninhydrin, and iodine vapors. Through most of the week the reaction was a heterogeneous mixture, with the starting material as oil which was denser than water. After one week the oil starting material was gone, although still visible on TLC. The reaction was removed from heat and allowed to cool to room temperature, and then was refrigerated to crystallize starting material. The crystallized starting material was filtered. Filtrate was evaporated and it was dried over P₂O₅ and NaOH to remove all water and [(HCl)]HCl. The crude product was washed with two portions of diethyl ether (50 ml each) to remove all starting material. It was again dried over P₂O₅. Yield: 2.83 g (45%). ¹H NMR (D₆-DMSO): δ

AF 1.33 (d, CH_3 , 3H); δ 2.92 (m, $N-CH_2$, 2H); δ 3.12 (m, SH , 1H); δ 3.18 (m, R_3-CH , 1H); δ 8.23 (bs, NH_3 , 3H). Melting point: 80-82°C (lit: 92-94).

AG On page 47, please replace the paragraph starting on line 12 with the following:

Ethylene diamine (42 μ l, 0.63 mmol, 5 fold excess), and pyridine (200 μ l, were added in CHC_1_3 (1 ml). 2-disteroyl-sn-p-nitrophenyl carbonate (100 mg, 0.13 mmol) was dissolved in $CHCl_3$ (1 ml) and added dropwise to ethylene diamine solution with a pastuer~~pasteur~~ pipette at 0°C (ice water) and continued overnight (16 h). TLC (CHC_1_3 : MeOH: H_2O 90: 18: 2, and CHC_1_3 : MeOH = 90: 10) showed that the reaction was complete. Solvent was evaporated to remove pyridine. Then the product mixture was dissolved in CHC_1_3 , loaded onto the column (Aldrich, Silica gel, 60°A, 200-400 mesh), and eluted with CHC_1_3 : CH_3COCH_3 , and CHC_1_3 : MeOH gradient, CHC_1_3 : CH_3COCH_3 = 90: 10, 60 ml (upper spot eluted); CHC_1_3 : NeOH = 90: 10, 60 ml (product eluted). Fractions containing pure product were combined and evaporated. Tert-butanol was added and dried in *vacuo* over P_2O_5 . Yield: 64 mg (75%). 1H NMR ($DMSO-d_6$, 360 MHz) δ .83 (t, end CH_3 , 6H); 1.22 (s, 28 \times CH_2 , 56H); 1.51 (m, CH_2CH_2CO , 4H); 2.25 (2xt, CH_2CO , 4H); 2.83 (m, $H_2NCH_2CH_2NH$, 2H); 3.21 (m, $H_2NCH_2CH_2NH$, 2H); 4.10-4.14 (m & cis d, $COOCH_2CHCH_2$, 4H); 5.17 (m, $OCOOCH_2CH$, 1H); 7.78 (m, $H_2NCH_2CH_2NH$, 2H).

AG On page 49, please replace the paragraph starting on line 4 with the following:

AG FGF or folate ligands were conjugated to maleimide-PEG-DSPE, according to procedures known in the art (Gabizon, A. et al, *Bioconjugate Chem.*, 10:289 (1999)). DNA-liposome complexes were incubated with micellar solutions of mPEG-DSPE, FGF-PEG-DSPE or folate-PEG-DSPE with continuous stirring for 20 minutes to achieve insertion of the ligand-PEG-lipid into the pre-formed liposomes.

On page 49, please replace the paragraph starting on line 15 with the following:

A21
KB tumor cells (1 million cells) were inoculated subcutaneously to the flank of nude mice. The mice were fed a reduced folate diet to upregulate the expression of folate receptors on the KB tumor cells. This model was used for folate-conjugated liposome-DNA complexes to target tumor vasculature angiogenic endothelial cells.

On page 51, please replace line 3 with the following (additions in bold):

C. In vivo Administration

On page 51, please replace line 26 with the following (additions in bold):

B. Liposome Formulations

On page 55, please replace line 7 with the following (additions in bold):

B. Liposome Formulations

On page 58, please replace line 12 with the following (additions in bold):

B. Liposome Formulations

On page 61, please replace line 8 with the following (additions in bold):

B. Liposome Formulations

On page 63, please replace the paragraph starting on line 6 with the following:

A22
Six-days after [(i)]implantation of Matrigel, 24 mice were randomized into treatment groups (n=3) for treatment with one of the formulations, Formulation No. (11-1) through Formulation No. (11-8). The liposome-DNA complexes were administered intravenously at a dose of 200 µg DNA plasmid. Twenty-four hours after administration of the FGF-targeted liposome-DNA complexes, luciferase expression in the matrigel, lung and liver was measured. The results are shown in Table 5.

On page 64, please replace line 1 with the following (additions in bold):

B. Liposome Formulations

On page 65, please replace the paragraph starting on line 4 with the following:

A23
Nine-days after inoculation with tumor cells, 15 tumor-bearing mice were randomized into treatment groups (n=3) for treatment with one of nine formulations, Formulation No. (12-1) through Formulation No. (12-5). The liposome-DNA complexes were administered intravenously at a dose of 100 µg DNA plasmid. Twenty-four hours after administration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4, luciferase expression in the tumor, lung and liver was measured. The results are shown in Table 6, where expression was measured 24 hours after liposome adminstration of Formulation Nos. 12-1, 12-3 and 12-5, and five days after administration of Formulation Nos. 12-2 and 12-4.

On page 65, please replace line 23 with the following (additions in bold):

B. Liposome Formulations

On page 68, please replace line 18 with the following (additions in bold):

B. Liposome Formulations

On page 70, please replace line 18 with the following (additions in bold):

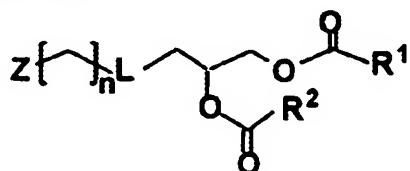
B. Liposome Formulations

Amendments to the Claims

1. (Currently amended) A composition for administration of a nucleic acid, comprising:

(a) liposomes comprised of

(i) a lipid having the formula



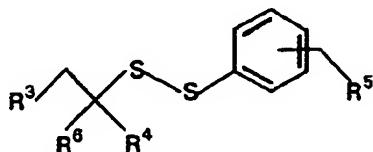
where each of R¹ and R² is an alkyl or alkenyl chain having between 8-24 carbon atoms, and each of R¹ and R² are independently selected;

n = [[1]]0-20;

L is selected from the group consisting of (1) -X-(C=O)-Y-[[CH₂-]], (2) -X-(C=O)-, and (3) -X-[[CH₂-]], where X and Y are independently selected from oxygen, NH and a direct bond;

Z is a weakly basic moiety that has a pK of less than 7.4 and greater than about 4.0; and

(ii) a compound having the general structure:



wherein R³ is a hydrophilic polymer comprising a linkage for attachment to the dithiobenzyl moiety; R⁴ is selected from the group consisting of H, alkyl and aryl; R⁵ is selected from the group consisting of O(C=O)R⁷, S(C=O)R⁷, and O(C=S)R⁷; R⁷ comprises an amine-containing lipid; and R⁶ is selected from the group consisting of H, alkyl and aryl; and where orientation of CH₂-R⁵ is selected from the ortho position and the para position; and

(b) a nucleic acid associated with said liposomes.

2. (Original) The composition of claim 1, wherein X is NH and Y is oxygen.
3. (Currently amended) The composition of claim 1, wherein L is a carbamate linkage, an ester linkage, or a carbonate linkage.
4. (Currently amended) The composition of claim 1, wherein L is NH-(C=O)-O[[-CH₂]].
5. (Original) The composition of claim 1, wherein Z is an imidazole.
6. (Original) The composition of claim 1, comprising between 1-80 mole percent of the lipid.
7. (Original) The composition of claim 1, wherein Z is a moiety having a pK value between 5.0-6.5.
8. (Original) The composition of claim 1, wherein each of R¹ and R² is an unbranched alkyl or alkenyl chain having between 8-24 carbon atoms.
9. (Original) The composition of claim 8, wherein each of R¹ and R² is C₁₇H₃₅.
10. (Original) The composition of claim 1, wherein n is between 1-10.
11. (Original) The composition of claim 1, wherein R⁶ is H and R⁴ is selected from the group consisting of CH₃, C₂H₅ and C₃H₈.
12. (Original) The composition of claim 1, wherein the amine-containing lipid comprises either a single hydrocarbon tail or a double hydrocarbon tail.

13. (Original) The composition of claim 1, wherein the amine-containing lipid is a phospholipid having a double hydrocarbon tail.

14. (Original) The composition of claim 1, wherein R⁴ and R⁶ are alkyls.

15. (Original) The composition of claim 1, wherein R³ is selected from the group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropyl-methacrylamide, polymethacrylamide, polydimethyl-acrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, polyaspartamide, copolymers thereof, and polyethyleneoxide-polypropylene oxide.

16. (Original) The composition of claim 1, wherein R³ is polyethyleneglycol.

17. (Original) The composition of claim 16, wherein R⁶ is H and R⁴ is CH₃ or C₂H₅.

18. (Original) The composition of claim 1, wherein said liposomes include between 5-20 mole percent of the compound.

19. (Original) The composition of claim 1, further including a therapeutic compound entrapped in the liposomes.

20. (Original) The composition of claim 1, wherein said nucleic acid is entrapped in at least a portion of said liposomes.

21. (Original) The composition of claim 20, wherein the nucleic acid is selected from DNA, RNA, fragments thereof and oligonucleotides.

22. (Original) The composition of claim 1, further including a ligand for targeting the liposomes to a target site, said ligand covalently attached to a distal end of the hydrophilic polymer R³ on said compound.

23. (Original) The composition of claim 22, wherein the ligand has binding affinity for endothelial tumor cells for internalization by such cells.

24. (Original) The composition of claim 22, wherein the ligand is selected from the group consisting of E-selectin, Her-2 and FGF.

25. (Original) The composition of claim 22, wherein said ligand is selected from the group consisting of c-erbB-2 protein product of the HER2/neu oncogene, epidermal growth factor (EGF) receptor, basic fibroblast growth receptor (basic FGF) receptor, vascular endothelial growth factor receptor, E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, CD19 receptor, αβ integrin receptors, and chemokine receptors.

26. (Original) The composition of claim 1, wherein said liposomes further comprise a cationic lipid.